to recall the classical experiments of Paul Bert in 1884 in which mastectomy of a pregnant goat led, at parturition, to the transitory appearance of a diabetic condition. Here the removal of the means whereby the physiological process could realize itself led to the pathological condition of a temporary diabetes.

If, in diabetic women, the large babies are to be ascribed to the action of an excessive secretion of growth hormone, I would suggest that the accidents of pregnancy in such women are more likely to be the result of other factors—perhaps an abnormality of secretion of pituitary gonadotropins

Conclusion

In a paper published some years ago (Young, 1947) I wrote: "Growth, including foetal growth, and milk production are processes requiring a special type of metabolic control in that they both necessitate the preservation from oxidization of foodstuff that would otherwise, in an animal in equilibrium, be oxidized. . . . Since growth, galactopoiesis, and diabetogenesis all involve a restraint on oxidative processes, it would not be surprising if there were some relationship between the hormonal mechanisms concerned with the control of these phenomena."

The evidence now at our disposal allows us to associate growth, including foetal growth, and milk production as phenomena whose development is stimulated by growth hormone. Excessive pressure on these processes in circumstances where the physiological stimulus cannot fully or properly realize itself can lead to the pathological outcome of diabetes, a condition in which the material preserved from oxidation but not utilized may be excreted in the form of sugar. In the human being a slightly excessive rate of secretion of growth hormone, insufficient to cause overt signs of acromegaly but lasting a long time, might lead to a prolonged but not excessive restraint upon the processes of carbohydrate and protein catabolism and oxidation, combined with the increase in appetite characteristic of growth-hormone activity, so that diabetes ultimately develops. In growth hormone we have a substance that may well be of importance in the aetiology of a serious proportion of cases of human diabetes mellitus.

I wish to express my thanks to the Medical Research Council for their continued support of my researches upon experimental diabetes.

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HYALURONIDASE SKIN SPREADING EFFECT*

AN ANALYSIS OF REPEATED MEASUREMENTS

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Hyaluronic acid has been demonstrated in man in the skin, umbilical cord, synovial fluid, vitreous humour, and some mesenchymal tumours. Its presence is inferred in connective tissue (Wislocki, Bunting, and Dempsey, 1947) on histological evidence and by observation of the effect of hyaluronidase. This enzyme system, found in some bacteria and in testicular extract, reduces the viscosity of, and hydrolyses, hyaluronic acid (Meyer *et al.*, 1937, 1940). The possible role of hyaluronic acid and hyaluronidase in connective-tissue diseases has been the subject of wide interest. Guerra (1946a, 1946b) claimed that the skin of patients with acute rheumatism gave enhanced spreading with the enzyme, and, further, that administration of salicylates inhibited this spread. Subsequently reported work (Harris and Friedman, 1949; Jaworski *et al.*, 1950) has not confirmed increased spreading in rheumatic fever, while recent work on the effect of salicylates gives conflicting results. Most reports agree that, *in vitro*, high concentrations are necessary to inhibit hyaluronidase; but while inhibition in therapeutic doses *in vivo* has been confirmed by some workers (Meyer, 1947; Dorfman *et al.*, 1947) it is denied by others (Swyer, 1948; Jones, 1950). Recent American literature (Symposium, 1950) favours the view that salicylates are inhibitory *in vivo*, but does not confirm the enhanced effect of spreading factor in rheumatic fever.

The well-known ability of haemolytic streptococci to form hyaluronidase (Meyer *et al.*, 1940; McClean, 1941; Crowley, 1944; Pike, 1948) has focused interest on the bacteriological relationship of this enzyme to rheumatic fever; the introduction of A.C.T.H. and cortisone, with their observed effect on connective tissue, redirects attention to the substrate and its place in the aetiology of this group of diseases. Preliminary reports indicate that in animals (Opsahl, 1949; Winter and Flataker, 1950) and in man (Shuman and Finestone, 1950) administration of these hormones causes decreased

*Based on a paper read at the Medical Research Society on July 6, 1951.

skin spread with hyaluronidase, and that local intra-articular cortisone increases the viscosity of joint fluid in rheumatoid arthritis (Bywaters and Dixon, 1951).

It seemed that there was no critical analysis of skin spread applicable to clinical research, although adequate investigations have been made on animals (Humphrey, 1943). The present work attempts to evaluate a suitable clinical method for detecting quantitative changes in skin response to hyaluronidase over a period of weeks, and describes some results in rheumatic patients undergoing hormone or salicylate treatment. A subsequent paper will describe investigations of the rate of reconstitution of the skin hyaluronic acid following enzyme treatment. Throughout the investigation bovine testicular hyaluronidase was used.*

Preliminary Investigations

The objects of this investigation were: (a) to establish a method of measuring spreading action in the skin repeatedly over a number of days or weeks and to estimate its variability; and (b) to observe the response in acute rheumatic fever, and note the effects of hormone or other therapy.

Indicator of Spread

Many substances have been suggested as indicators of degree of spread, but a number of them inhibit hyaluronidase, and this severely limits the choice among dyes and coloured substances. Indian ink, Congo red (0.1% and 0.01%) and Evans blue (0.1% and 0.01%) all produced prolonged or permanent skin staining; various blood pigment derivatives (Fulton *et al.*, 1948) and haematin inhibited hyaluronidase. It has been suggested (Shuman and Finestone, 1950) that *fluorescein* may be used as an indicator by observing under a Wood's lamp the "disappearance-time" of fluorescence in the skin following intradermal injection with the enzyme; but clinically the test was too time-consuming and laborious. Furthermore, it was shown† that a 2.5% solution of fluorescein completely inhibited hyaluronidase, although the concentration used in the test (1/50,000) appeared not to affect the enzyme *in vitro*.

Indigo carmine (0.5% in isotonic PO_4 -buffered saline, pH 7.2) was painless on injection, gave a clearly defined blue stain capable of accurate measurement, and disappeared within three days. Viscosimetric determinations† of its hyaluronidase inhibitory activity, however, showed that it caused 72% inhibition of a standard hyaluronidase solution within five minutes of mixing. Although the remaining activity caused measurable spread, this was a variable factor, and an analysis of variance of the measure of spread in tests on three individuals on three days each showed a significant component of variation due to differences in test procedure.

Haemoglobin.—Although haemoglobin solution has been widely used in animals and man as a spreading indicator (Madinaveitia, 1938; Humphrey, 1943; Harris and Friedman, 1949; Jones, 1950) its use in repeated skin tests in man seemed not without danger of producing sensitization to blood-group substances. The likelihood of this was minimized by using Group O rhesus-negative blood as a source, although this limited available supplies. Viscosimetric estimations† showed that it had no effect on hyaluronidase activity, and the

skin stain disappeared in two to four days. In the absence of a more easily prepared indicator, haemoglobin was adopted and proved satisfactory in use.

Method for "Spreading Test"

General.—The flexor forearm skin was used in all subjects. Superficial veins were avoided so far as possible, because the indicator tended to spread along them, with resultant irregular areas of stained skin, making accurate measurement difficult. All-glass siliconed Mantoux syringes (Luer) with short-bevelled 26 B.W.G. needles were used, and large batches of enzyme and indicator avoided changes of test solutions.

Indicator Solution.—Sterile isotonic human haemoglobin solution with a pH of 7.2 was prepared from Group O rhesus-negative blood by lysing packed red cells with an equal volume of distilled water and carefully dissolving in the resulting haemoglobin solution a mixture of 1.65 g. % of $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ and 0.103 g. % of KH_2PO_4 . The solution was run through a clarifying filter to remove stroma and then through a sterilizing pad. It was stored at 4°C., and remained stable for several weeks. The haemoglobin content of this product was 12.4 g. %.

Enzyme.—Bovine testicular hyaluronidase in the form of a dried commercial preparation ("hyalase") containing 1,000 "Benger units" per ampoule.

Intradermal injections.—The contents of 1 ampoule of desiccated hyalase dissolved in 1 ml. of indicator constituted the test solution, whereas the haemoglobin alone acted as the control. Attention was paid to the mechanics of intradermal injection (Hechter, 1947); all the injections were performed by the same observer to ensure, so far as was possible, that exact volumes and comparable plunger pressures were used. Siliconed syringes facilitated smooth plunger action with the rather sticky haemoglobin solution.

Dose and Measurement.—The test and control solutions, 0.1 ml. of each, were injected intradermally. Maximum and minimum diameters were measured to the nearest millimetre with needle-pointed callipers, and their product was multiplied by $\pi/4$. This gave the area of the ellipse in square millimetres. Readings

FIG. 1.—Eighteen "spreading tests" on three normal adults 65, 70, and 80 minutes after injection. Tests on inner (L.) and controls on outer (R.) aspects of forearms.

*Supplied by Benger's Ltd., Research Department.

†Performed by Benger's Ltd., Research Department.

repeated at 30 and 60 minutes showed a difference in spread, but the stained area was more clearly defined at the end of one hour (see Fig. 1). The purpose of this investigation was to measure gross differences in spreading in patients on various therapeutic regimes. In testing fairly large numbers of patients it was not practicable to measure spreading areas after only, say, five minutes, as advocated by Hechter (1950). Readings were therefore made at one hour, in agreement with the practice of other clinical workers (Harris and Friedman, 1949; Jones, 1950), and the degree of spread expressed as the "spreading ratio" (test/control ratio, or T/C ratio).

The first experiment was designed to discover whether there was a significant difference between skin sites on the same day or between repeated tests on different days.

Experiment 1

Three normal adult subjects (two male and one female) had six test and six control injections into the flexor skin of one forearm, the medial side being used for the test and the lateral side for the control injections (Fig. 1). This procedure was repeated on four different days, alternate forearms being used, allowing an interval of two to five days between tests and hence adequate time for reconstitution of the hyaluronic acid depolymerized by the previous enzyme injections. On each day identical solutions were used, fresh enzyme was dissolved, and the injections were performed and measurements made by the same observer (E. J. H.).

Results.—Two of the three subjects showed sensitization (flare with or without wheal) to the enzyme preparation during repetition of tests. They were therefore excluded, and the subject not exhibiting sensitivity gave the results shown in Table I—which shows that variation

TABLE I.—Experiment 1. Day-to-day Variation in Spreading Test
—Spreading (T/C) Ratio in 6 Tests, Repeated on 4 Days in One Subject

Skin Site	1st Day	2nd Day	3rd Day	4th Day
1	1.96	2.66	1.54	2.70
2	1.87	2.58	1.91	2.00
3	1.95	2.65	2.65	2.23
4	1.95	2.33	1.98	2.17
5	2.07	2.28	1.73	2.10
6	1.88	2.56	1.79	1.75

Total degrees of freedom = 23.

Analysis of Variance

Item	Sum of Squares	n	Mean Sq.	Variance Ratio	P
Sites	0.3794	5	0.0759	1.14	Not significant
Days	1.3037	3	0.4344	6.48	0.01
Error	1.0080	15	0.0672	—	
	2.6911				

in spreading ratios on different days was not due to differences in spreading between skin sites. Thus one pair of injections sufficed on any part of the flexor forearm skin.

Experiment 2

This experiment was made to determine the range of variation from day to day. Six normal adults (four male and two female) had one pair of injections on each of four different days. Identical preparations were used for all subjects on each day, and the injections performed and measurements were made by one observer (E. J. H.).

Results.—Two subjects showed sensitivity during repetition of the tests and were therefore excluded from analysis of the results given in Table II, which shows

TABLE II.—Experiment 2. Measurement of Day-to-day Variation
—Spreading (T/C) Ratio in 4 Normal Adults Tested on 4 Different Days

Subject	1st Day	2nd Day	3rd Day	4th Day
G.L. ..	2.92	2.01	2.15	2.77
M.S. ..	1.37	2.04	2.37	1.65
L.G. ..	2.94	2.17	2.04	2.17
R.C. ..	2.00	1.96	2.52	2.64

Analysis of Variance. Total No. of Tests, 16

Item	Sum of Squares	n	Mean Sq.	Variance Ratio	P
Individuals ..	0.8211	3	0.2737	1.14	Not significant
Days	0.1912	3	0.0637	—	" "
Error	1.8672	9	0.2075	—	" "
	2.8795	15			

Variance = 0.191967. Mean = 2.23. S.E. = 0.45. Variance of mean = 0.011997.

that: (1) day-to-day variation is due to individual characteristics and not to variations in technique; (2) differences observed between individuals are not significantly greater than day-to-day variation in single individuals; and (3) the coefficient of variation is about 20%.

Experiment 3

This experiment was made for the purpose of observing the range and mean spreading ratio in a group of rheumatic fever cases. Initial and acquired hypersensitivity to the enzyme preparation presented a considerable difficulty in the interpretation of readings, and is discussed below. To exclude hypersensitivity effects in the estimate of range and mean in rheumatic fever subjects, readings in this experiment were taken only from subjects undergoing their first enzyme (spreading) test, and cases showing wheal, flare, or indefinite margin of spread area were excluded. Twenty-one cases of acute rheumatism gave tests satisfying these criteria; in all there was a recent history of arthritis and signs of active carditis with raised E.S.R. The results are shown in Table III, and are compared with normal subjects in Table IV. It will be seen that the normal mean is 2.23,

TABLE III.—Experiment 3. Spreading Test in Acute Rheumatic Fever. Spreading (T/C) Ratio in 21 Cases of Acute Rheumatic Fever (One Test Only on Each Case)

2.08	2.65	1.85	1.62	1.96	1.98	2.38
1.71	1.42	1.65	1.49	1.53	1.61	2.61
2.36	2.80	2.60	2.56	2.08	1.81	2.72

Variance = 0.209580. Mean = 2.07. S.E. = 0.46. Variance of mean = 0.009980.

TABLE IV.—Comparison of Results in Normal Subjects (Experiment 2) and in Cases of Acute Rheumatic Fever (Experiment 3)

Experiment 2 ..	Mean, 2.23	Variance of mean, 0.011997
" 3 ..	" 2.07	" " " 0.009980
	Difference, 0.17	Sum, 0.021977

S.E. of difference = 0.148
c = 1.15. P = 0.25. (n = >30)

the rheumatic mean 2.07, and the S.E. of their difference is 0.148; the difference in this comparison is therefore not significant, which does not support Guerra's claim that skin spread is enhanced in patients with acute rheumatism.

Development of Hypersensitivity

Sherwood Jones (1950) reported a high incidence of initial allergic reactions in both normal and rheumatic fever subjects. In the present investigation a somewhat purer enzyme preparation was used, and immediate local reactions were fewer, although, as shown in Table V,

TABLE V.—*Hypersensitivity in "Spreading Test"*

No. of Tests Before Sensitivity Acquired	No. of Cases (Both Sexes and All Ages)			Total No. of Cases	Total No. of Tests	Final Grade of Sensitivity	
	R.F.	R.A.	C.D.			No. Cases	Grade
0	11	3	3	17	101	{ 7 10	1 2
1	7	2	—	9	77	{ 4 5	1 2
1-5	6	1	1	8	85	{ 3 5	1 2
5-10	4	2	—	6	67	{ 4 2	1 2
Over 10 ..	5	1	—	6	110	{ 3 3	1 2
Totals ..	33	9	4	46	440	{ 21 25	1 2

R.F. = Rheumatic fever. R.A. = Rheumatoid arthritis (adults and children). C.D. = Collagen diseases (excluding dermatomyositis).

FIG. 2.—(a) A "normal" spreading test, the test injection being the same depth of colour as the control, with a small pale central area and a bluish clearly defined rim of pigment. (b) Grade 1 hypersensitivity. The test area is large, pale, homogeneous, without a pale central spot, and with an indefinite edge incapable of accurate measurement. (c) Grade 2 hypersensitivity. The test injections are invisible owing to complete dispersal of the haemoglobin within the hour. (d) Grade 2 hypersensitivity. The test injections show a wheal and flare.

eventually all patients became sensitized; this sensitivity may probably be ascribed to protein impurity in the enzyme preparation. Non-specific serum inhibitor was not measured in this study.

A normal (non-sensitized) reaction consisted of a test injection with the same depth of colour as the control, a small pale central area, and a bluish clearly defined rim of pigment (Fig. 2, a). When sensitivity occurred the test area became large and pale, homogeneous, without a pale central spot, and with an indefinite edge incapable of accurate measurement (Grade 1 sensitivity) (Fig. 2, b). With more marked reaction there was an immediate wheal and flare, or complete dispersal of the indicator within an hour, rendering the test invisible (Grade 2 sensitivity) (Fig. 2, c and d). Allergic dermal oedema potentiates enzyme spread owing to increased intradermal pressure (Hechter, 1947), so it was necessary to determine a spreading ratio value below which spread due to oedema could be reasonably excluded. The criteria of sensitivity described above were used, and a critical value of the spreading ratio of 3 was derived by taking the mean ratio of the 21 non-sensitized cases shown in Table III, and adding twice the standard error of the sample. Thus values larger than 3 were considered unlikely ($P = 0.02$) to occur in rheumatic fever cases without sensitization; and in fact values above 3 were usually associated with definite signs of hypersensitivity.

Table V shows the incidence of hypersensitivity. We tested 33 cases of rheumatic fever, 9 cases of rheumatoid arthritis, and 4 cases of collagen disease. The collagen disease group included 1 case of polyarteritis nodosa, 1 of erythema nodosum, and 2 of disseminated lupus erythematosus. These 46 cases had 440 tests with hyaluronidase and all eventually became sensitized, 21 to Grade 1 sensitivity, and 25 to Grade 2 sensitivity. One-third of these exhibited sensitivity at their first exposure to the substance, others after one injection, and some not until 10 or more tests had been performed. The proportion of hypersensitivity increased with serial testing, as did the degree of sensitivity in the same individual.

Dermatomyositis.—Three cases of dermatomyositis showed a different skin reaction, there being no macroscopic difference between test and control injections. One of these patients died before hypersensitivity developed, but the others eventually became sensitized (Table VI). This suggests that the substrate is altered in this disease, preventing hyaluronidase spread.

TABLE VI.—*"Spreading" Test in Dermatomyositis*

Sex	Age	Total No. Tests	Results
F	8	11	Practically no difference between test and control injections. Died before hypersensitivity occurred
F	13	34	At first no difference between test and control. Then slight difference followed by Grade 2 sensitivity
F	19	8	No difference between test and control followed by Grade 1 sensitivity

Experiment 4: The Effect of Hormone Treatment on the Spreading Test

Acute rheumatic fever cases were allocated at random to one of three treatment groups—salicylate, cortisone, or adrenocorticotrophic hormone (A.C.T.H.). The treatment lasted six weeks, the total hormone dosage for

each patient being fixed at 4.1 g. of cortisone or 1.62 g. of A.C.T.H., irrespective of age or body weight. Salicylate, however, was given as aspirin, 1-4 g. daily, according to body weight. This small dosage did not always control acute arthritis, which sometimes required a supplement of pethidine. Fig. 3 shows the effect of therapy on skin spread, each patient having proved rheumatic activity at the start of treatment. Four spreading tests were done on each case at approximately equally spaced intervals during treatment.

Results.—In the salicylate group the readings either fell within normal range or exhibited sensitivity. Some of the cortisone cases showed inhibition of spread, but only the A.C.T.H. group showed a significant difference, the mean of all the readings lying below the normal range. In the salicylate group 12 out of 22 tests showed hypersensitivity; with cortisone 8 out of 24 and with A.C.T.H. only 2 out of 24 tests showed hypersensitivity during treatment. However, all the A.C.T.H. group showed a marked "release" rise of ratio following cessation of therapy.

Discussion

Haemoglobin was found to be the best of a number of substances tested as spreading indicators, and gave as clear a zone of staining as any of the chemical dyes tested. None of the latter were satisfactory as indicators, mainly because of their inhibitory effect. The tonicity and pH of the haemoglobin solution, which profoundly affect the activity of the enzyme (McClellan and Hale, 1941; Madinaveitia and Quibell, 1941; McClellan, 1943; Hale, 1944), required careful adjustment to avoid pain on injection. The pH chosen (7.2) lies within the range allowing hyaluronidase activity.

When the spreading test was used repeatedly in the same patient hypersensitivity always developed, and this proved a serious drawback to the usefulness of the test. It is possible that with purer preparations of bovine hyaluronidase this effect might be delayed or reduced. The incidence of tests showing hypersensitivity in the three groups suggests an inhibitory effect on this phenomenon by A.C.T.H.; although the numbers in each treatment group were small all patients eventually showed hypersensitivity after treatment was stopped, including the A.C.T.H.-treated group. The effect of cortisone on development of hypersensitivity is probably not significant; this is in agreement with Stollerman *et al.* (1951), who found that cortisone therapy did not inhibit the wheal and erythema resulting from union of antigen and sensitizing antibody in the skin of human beings. In the salicylate group development of hypersensitivity was observed in all six cases; but, as already stated, the dosage was rather low.

Even with the exclusion of hypersensitivity effects, the spreading ratio showed considerable variation in one individual from day to day; in studying groups of cases, however, this effect was balanced by the similar

FIG. 3.—Effect of therapy on spreading action and sensitizing effect of bovine testicular hyaluronidase in acute rheumatic fever.

variation observed between patients (as shown in Table II), so that the effect on spreading ratio of drugs used in the treatment of groups of cases was discernible by comparison of mean values in different groups. Variation was observed, as described, both between individuals and from day to day in the same individuals. Differences between individuals may be ascribed partly to age and sex (Lurie and Zappasodi, 1939); in children and women, spreading was more clearly defined than in men, and in atrophic inelastic senile skin.

The occurrence of day-to-day variation in an individual was shown to be due to causes other than variation in technique—that is, there was a real individual variation. Environmental conditions may contribute to this, but humoral factors also may be significant. For example, in rabbits oestrogens (Sprunt and McDearman, 1940) inhibit spread, whereas Lurie and Zappasodi (1942) found an opposite effect with luteinizing hormones. With regard to non-specific serum hyaluronidase inhibitor, although this has been shown by Dorfman (1950) to be increased in rheumatic fever, it has also been shown (Dorfman and Moses, 1950) that A.C.T.H. decreases its serum level in this disease. The decreased spreading effect associated with A.C.T.H. described above is therefore unlikely to be due to action by the inhibitor, and may reasonably be ascribed to the action of the hormone. This is in agreement with Hechter (1947), who concluded that serum anti-hyaluronidases do not significantly affect the spreading activity of the injected enzyme. It is known that A.C.T.H. stimulates the production of a number of corticosteroids, including compound F, and some of these have been shown to be hyaluronidase inhibitors in animals (Opsahl, 1949; Winter and Flataker, 1950). Alternatively, adrenal corticosteroids may alter the substrate in such a way that the spreading effect of hyaluronidase is reduced.

The findings in dermatomyositis are interesting; hyaluronidase seemed to cause no significant spreading effect in the skin of these cases, unless there was also hypersensitivity to the enzyme. In this disease fibrosis and oedema in the skin are possible factors influencing spread. In oedema of renal or cardiac origin we have not found reduced spreading; and in six individuals with new or old scars injection of haemoglobin and

hyaluronidase into scar tissue showed considerably greater spread than haemoglobin alone. Our findings suggest, therefore, that in dermatomyositis the hyaluronic component of the skin is either deficient or is unresponsive to testicular hyaluronidase.

Summary

A method, using haemoglobin as indicator, of measuring the spreading effect of hyaluronidase in the skin on repeated occasions is described and its inherent variability assessed.

This "spreading test" was performed in cases of rheumatic fever, rheumatoid arthritis, and connective-tissue disease.

No significant difference was found in hyaluronidase spread in patients with acute rheumatic fever and in normal subjects.

Hypersensitivity to the enzyme preparation develops readily; this limits the usefulness of the method.

Significant inhibition of spread and of hypersensitivity to hyaluronidase occurs in the skin of rheumatic fever patients under treatment with A.C.T.H.

In dermatomyositis no significant spreading effect was observed with hyaluronidase.

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The Scottish National Blood Transfusion Association announces that in the last 12 months about 55,000 pints of blood were used in Scottish hospitals. There are 10 Scottish donors who have given more than 50 donations, and 102 who have given more than 25 donations.

RECONSTITUTION OF THE DERMAL BARRIER TO DYE SPREAD AFTER HYALURONIDASE INJECTION*

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The spread of material injected into the skin depends upon the state of the ground substance. Hyaluronidases depolymerize and hydrolyse the hyaluronic acid component, with resulting increase in spread. Subsequently the hyaluronic acid is replaced or restored to its original state, and the barrier to spread is reconstituted.

Hechter (1948) has investigated the time taken for reconstitution of this barrier after breakdown by hyaluronidase, using the disappearance time of saline blebs in skin sites previously treated with testicular enzyme; he concluded from results with a range of enzyme concentrations that restoration of the barrier took from 24 to 48 hours in six normal adults. No other work in this field has been found by us. Hechter infers that the replacing substance is identical with the original substrate, since it reacts to a further dose of enzyme in the same manner as untreated skin.

The role of hyaluronic acid in the pathogenesis of rheumatic fever is not known. In the joint fluid from acute rheumatism the viscosity is only slightly decreased, whereas in rheumatoid arthritis defective polymerization of the hyaluronic acid of the synovial fluid is indicated by a well-marked fall in viscosity (Ragan and Meyer, 1949). Furthermore, intra-articular (local) cortisone in this disease causes the secretion of normal (highly polymerized) hyaluronic acid with increase in viscosity of the fluid (Bywaters and Dixon, 1951). However, the histology of rheumatic fever shows abnormal Feulgen staining of the connective tissue in and around lesions; and the present work presents evidence which suggests alteration in the reconstitution rate of skin hyaluronic acid after its breakdown by hyaluronidase in cases of acute rheumatic fever and rheumatoid arthritis and some observations of the effects of A.C.T.H. and cortisone on this abnormal response.

Methods

Skin subjected to hyaluronidase action continues for a time to permit increased spreading of injected material. This has been explained on the hypothesis that the hyaluronic acid depolymerized by the enzyme is itself repolymerized or replaced by more highly polymerized substrate, processes which take time to complete. Serial

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